# PATENT COOPERATION TREATY



From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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# PCT

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
*(day/month/year)* see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

**FOR FURTHER ACTION**
See paragraph 2 below

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US2004/020336 | 23.06.2004 | 23.06.2003 |

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68, C07H21/00

Applicant
EPIGENOMICS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2004/020336

**Box No. I Basis of the opinion**

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 9,20,45(in full),44(for IA),1-8,10-19,21-44,46-51(in part)

because:

☒ the said international application, or the said claims Nos. 44 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

**see separate sheet**

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos.. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 9,20,45(in full),1-8,10-19,21-44,46-51(in part)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

| | | |
|---|---|---|
| the written form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |
| the computer readable form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

## Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   **see separate sheet**

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-51(in part)

## Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 2-8,10-19,21-44,51 |
| | No: | Claims | 1,46-50 |
| Inventive step (IS) | Yes: | Claims | 2-8,10-19,21-44 |
| | No: | Claims | 1,46-51 |
| Industrial applicability (IA) | Yes: | Claims | 1-8,10-19,21-43,46-51 |
| | No: | Claims | |

2. Citations and explanations

   **see separate sheet**

## Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

Reference is made to the following documents:

D1: WO 02/24056 A (MARKOWITZ SANFORD ; UNIV CASE WESTERN RESERVE (US); GRADY WILLIAM (US)) 28 March 2002 (2002-03-28)

D2: WO 03/014388 A (TAUBERT HEIKE ; DISTLER JUERGEN (DE); EPIGENOMICS AG (DE); MODEL FABIA) 20 February 2003 (2003-02-20)

D3: TOYOTA M ET AL: "CpG island methylator phenotype in colorectal cancer" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, July 1999 (1999-07), pages 8681-8686, XP002307651 ISSN: 0027-8424

D4: VAN RIJNSOEVER M ET AL: "Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands." GUT, vol. 51, no. 6, December 2002 (2002-12), pages 797-802, XP002328020 ISSN: 0017-5749

D5: RASHID ASIF ET AL: "CpG island methylation in colorectal adenomas" AMERICAN JOURNAL OF PATHOLOGY, vol. 159, no. 3, September 2001 (2001-09), pages 1129-1135, XP002328021 ISSN: 0002-9440

D6: DATABASE EMBL [Online] 15 May 2003 (2003-05-15), "Homo sapiens SLIT and NTRK-like family, member 1, mRNA (cDNA clone MGC:51091 IMAGE:4816570), complete CDs." XP002328022 retrieved from EBI accession no. EM_HUM:BC051738 Database accession no. BC051738

D7: DATABASE EMBL [Online] 17 September 2001 (2001-09-17), "Homo sapiens mRNA for KIAA1910 protein, partial CDs." XP002328023 retrieved from EBI accession no. EM_HUM:AB067497 Database accession no. AB067497

Section III

1 Claims for which no international search report has been established have not been examined (cf Rule 66.1 PCT). Therefore, no opinion is provided with respect to the provisions of Art.33(1) PCT (i.e. novelty, inventive step and industrial applicability) for claims 9, 20 and 45, and claims 1-8, 10-19, 21-44 and 46-51 are examined only insofar as they relate to SEQ ID Nos 1, 304, 305, 420 and 421.

2 In between claims 43 and 44 is a claim numbered 1; said claim (hereinafter referred to as claim 43b) is a dependent claim referring to claims 40 and 45, yet claim 45 is

directed to a product. Therefore, claim 43b is unclear (Art.6 PCT) and has been searched and is examined only insofar as it refers to claim 40.

4 Claim 44 refers to a method comprising a step of obtaining a biological sample from a subject: the method is considered to encompass a surgical step to be carried out on the human body and is thereby covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

Section IV

1 The application lacks unity within the meaning of Rule 13.1 PCT and relates to the following (groups of) inventions:

Invention 1: Claims 1-51 (all partially)

A nucleic acid comprising at least 9 or 16 contiguous nucleotides of a "treated" DNA sequence consisting of SEQ ID No.304, 305, 420 or 421 or sequences complementary thereto; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising contacting genomic DNA with (a) reagent(s) that distinguish methylated and non-methylated CpG dinucleotides within at least one or at least two target region(s) of the genomic DNA that comprises at least one CpG dinucleotide sequence, in particular wherein said at least one target region comprises or hybridizes to at least 16 contiguous nucleotides of the sequence defined by SEQ ID NO.1; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising determining the expression levels at least of the gene defined by SEQ ID NO.1; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising the ability of a methylation-sensitive restriction enzyme to cleave a taget genomic DNA, wherein said target nucleic acid comprises or hybridizes to at least 16 contiguous nucleotides of the sequence defined by SEQ ID NO.1.

Invention 2: Claims 1-51 (all partially)

A nucleic acid comprising at least 9 or 16 contiguous nucleotides of a "treated" DNA sequence consisting of SEQ ID No.306, 307, 422 or 423 or sequences complementary thereto; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising contacting genomic DNA with (a) reagent(s) that distinguish methylated and non-methylated CpG dinucleotides within at least one or at least two target region(s) of the genomic DNA that comprises at least one CpG dinucleotide sequence, wherein said at least one target region comprises or hybridizes to at least 16 contiguous nucleotides of the sequence defined by SEQ ID NO.2; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising determining the expression levels at least of the gene defined by SEQ ID NO.2; a method for detecting and/or detecting and distinguishing between or among colorectal cell proliferative disorders comprising the ability of a methylation-sensitive restriction enzyme to cleave a taget genomic DNA, wherein said target nucleic acid comprises or hybridizes to at least 16 contiguous nucleotides of the sequence defined by SEQ ID NO.2.

Inventions 3-64: Claims: 1-51 (all partially)

As invention 2, for genomic sequences 3-64 and their corresponding "treated" sequences.

2 Note: The genes referred to in the claims are clear only insofar as they are defined by the genomic sequences given by SEQ ID Nos 1-64. Consequently, the listed inventions refer only to these sequences and not the gene names as given in the claims.

3 The above inventions are linked in that they all relate to genomic DNA sequences and modifications thereto which reflect the results of a treatment allowing distinctions to be made according to the methylation status of CpG dinucleotides in said sequences. According to the treatment, all non-methylated "C" bases are converted to "T", whereas methylated "C" bases remain unchanged. Thus, SEQ ID NOS 1-64 define genomic DNA sequences; SEQ ID NOs 304-419 and 65-76 define the corresponding sense and antisense strands in which all cytosines except those in a CpG dinucleotide have been converted to thymidines (i.e. all "C" residues of CpG dinucleotides are assumed to be methylated and are therefore not converted to "T");

SEQ ID NOs 420-535 and 77-88 define the corresponding sense and antisense strands wherein all cytosines have been converted to thymidines (i.e. all "C" residues, including those of CpG dinucleotides, are assumed to be non-methylated and are therefore converted to "T"). The inventions also relate to methods using said sequences for detecting and/or detecting and distinguishing colorectal cell proliferative disorders.

4 These links cannot be considered to provide a single inventive concept in the sense of Rule 13.2 PCT for the following reasons: methods for the analysis of colon cancer (i.e. a colon cell proliferative disorder) according to the methylation status of genomic DNA sequences are known in the art. Thus, D1 discloses methods for detecting cancers, in particular colon cancer, by determining the methylation status of CpG dinucleotides in the promotor region of the hMLH1 gene (D1: p.11, l.20 - p.13, l.11). D2 provides methods for analysis of colon cancer by determining the methylation status of CpG dinucleotides in a number of genes (cf claims 1, 2, 16). Moreover, both D1 and D2 use methodology in which genomic DNA is chemically treated in order to convert non-methylated cytosine bases to thymidine (or uridine) bases (D1: p.7, l.21 - p.9, l.2 and p.13, l.12 - p.15, l.27; D2: p.10, l.1 - p.16, l.19) and provide appropriate sequences of primers, probes, or the converted genomic sequences (e.g. D1: Table 1, Fig.2; D2: p.23, l.1 - p.24, l.8). Furthermore, D3, D4 and D5 (cf abstracts) discuss distinguishing between different colorectal tumour phenotypes (i.e. distinguishing colorectal cell proliferative disorders) according to their differing methylation states.

5 Therefore, in the light of the prior art, the problem to be solved by the present application is the provision of further DNA sequences and their use in methods for analysis of colon cell proliferative disorders according to the methylation status of the genomic DNA.

6 Each of the 64 groups of DNA sequences, each comprising one genomic DNA sequence and its four corresponding sequences post-treatment, provides a separate solution to the given problem, each group thereby giving rise to a different invention.

7 Due to the fact that the different groups of sequences do not share any significant common structural element other than the presence of CpG or corresponding

dinucleotides, that the only features which could be identified as representing a special technical feature in the sense of Rule 13.2 PCT are those identified above, and that no other feature could be identified which might be considered to represent a common inventive link between the subject-matters of the different inventions, the present application does not meet the requirements of Rule 13.1 PCT with respect to unity of invention, and is considered to consist of the 64 separate inventions given above.

8 A search has been established for Invention 1, which is the subject of this written opinion.

Section V

1 Novelty (Art.33(2) PCT)

1.1 The subject-matter of claim 1 is not novel over D1 or D2. D1 discloses methods for detecting cancers, in particular colon cancer, by determining the methylation status of CpG dinucleotides in the promotor region of the hMLH1 gene (D1: p.11, l.20 - p.13, l.11). D2 provides methods for analysis of colon cancer by determining the methylation status of CpG dinucleotides in a number of genes (cf claims 1, 2, 16). Moreover, both D1 and D2 use methodology in which genomic DNA is chemically treated in order to convert non-methylated cytosine bases to thymidine (or uridine) bases (D1: p.7, l.21 - p.9, l.2 and p.13, l.12 - p.15, l.27; D2: p.10, l.1 - p.16, l.19). Feature ii) of claim 1 is considered to be non-limiting (cf item VIII.1).

1.2 The subject-matter of claim 46 is not novel (Art.33(2) PCT) over D6 or D7, which define nucleic acid molecules comprising portions of 18 or more contiguous nucleotides that are identical to each of SEQ ID Nos 304, 305, 420 and 421, and which comprise at least one CpG, TpA or CpA dinucleotide. Similarly the subject-matter of claims 47-50 is not novel over D6 or D7.

1.3 The subject-matter of claims 2-8, 10-19, 21-44 and 51 appears not to be known from the prior art and therefore is novel (Art.33(2) PCT).

2 Inventive step (Art.33(3) PCT)

2.1 The prior art provides no indication as to a link between the CpG methylation status of a DNA molecule having a sequence defined by SEQ ID NO.1 (which corresponds with the human SLITRK1 gene, also known as KIAA1910: cf D6 and D7) and colorectal cell proliferative disorders, as disclosed in the present application. Therefore, the subject-matter of claims 2-8, 10-19 and 21-44 would appear to involve an inventive step as required by Art.33(3) PCT.

2.2 With respect to claim 51, not all of the nucleic acids and oligomers defined as in claims 46-50 would necessarily be suitable for detecting and/or distinguishing between colon cell proliferative disorders, particularly those lacking at least one CpG, CpA or TpG dinucleotide. The problem to be solved by claim 51 over D1 or D2 would be considered as the provision of alternative oligomers suitable for detecting and/or distinguishing between colon cell proliferative disorders. As said problem is not solved over the entire scope of claim 51, no inventive step can be acknowledged for claim 51.

3 Industrial applicability (Article 33(4) PCT)

For the assessment of the present claim 44 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Section VIII

1 Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter partly in terms of the result to be achieved, namely in part ii) referring to the

sensitivity and specificity of the detection, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

2 In claims 46 and 48, the feature "wherein the treatment..." is superfluous (Art.6 PCT), as said claims are directed to products, i.e. defined sequences, and the type of treatment that has been carried out in order to arrive at said sequences does not help to define the sequences per se.